

ON THE HEART OF THE ORANGE TUNICATE,
ECTEINASCIDIA TURBINATA HERDMAN

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Members of the genus *Ecteinascidia* belong to the Subphylum Tunicata. This subphylum is also referred to as the Urochordata (Urochorda), and the animals are commonly known as ascidians or sea-squirts. As a group, they are highly successful, diverse and specialized (not degenerate) protochordate members of the Phylum Chordata (Barrington, 1965). *E. turbinata* Herdman grows at various depths on exposed surfaces in dense, almost spherical, usually pinkish orange to reddish orange clusters of various diameters (colonies measure from about one to 7 inches), made of many sessile individuals. Many of the individuals in a colony are connected by short, delicate, branched stolons. *Ecteinascidia* belongs to the largest of the 3 classes of the Tunicata: to the Ascidiacea (Berrill, 1932; Van Name, 1945).

A very large literature is available on the hearts of many species of tunicates and most of the pertinent information on the anatomy and physiology of this organ has been well-reviewed by Skramlik (1929, 1938), Millar (1953), and Krijgsman (1956). The primary and perennial interest in the hearts of tunicates is the periodic reversal in the direction of the heart beat in all species of these quite diverse animals. The physiological usefulness (if any) of the periodically reversing circulation still remains unknown. Further, none of the various explanations of periodic reversal is entirely satisfactory (Mislin, 1969). The two major theories of causes of heart beat reversal in tunicates are, first, that back pressure gradually builds up within the vascular system to inhibit the active pacemaker and, second, that the active pacemaker "fatigues." Krijgsman (1956) concluded that regular reversal of pulsation could not be accounted for by the back pressure theory. He proposed that periodic fatigue and subsequent restoration of heart pacemakers offered a better explanation. Nevertheless, the fatigue theory is ill-defined and poses more questions than it solves. The phenomenon of heart reversal is not unique to tunicates since it is known to occur in at least 7 orders of insects (Jones, 1964) and has been observed in blood vessels of both vertebrates and invertebrates (Azariah, 1965; Mislin, 1969). Beklemishev (1969) refers to heart reversals in Nemertinea and in *Amphioxus*.

Although the hearts of many species of tunicates have been studied in varying detail, that of *Ciona intestinalis* has been studied more extensively and in far more detail than that of any other single species. Consequently, information on *Ciona* tends to dominate and outweigh that on other species. The present paper deals with observations made each summer over a five year period on the general anatomy and physiology of the heart of *Ecteinascidia turbinata* Herdman, 1880.

MATERIALS AND METHODS

Most specimens were collected from the pilings off Longbird bridge, just past the entrance to Castle Harbour in Bermuda. Usually within an hour of collec-

tion the colonies were secured at the bottoms of tanks of flowing sea water, some distance away from the slow steady inflow. Colonies also did moderately well when kept in large beakers of sea water which was changed each day. The water temperature varied from 25 to 28° C. Unless stated differently, healthy-looking tunicates were carefully torn from a colony with fine forceps and placed with their right sides uppermost in 10 ml of fresh sea water in small glass dishes. Normally, the animals live with their siphons uppermost, their posterior stolons attached to the substrate. When removed from the colony, the animals can move about considerably by currents set up by their siphons. Animals which were badly silted or in obviously poor condition were automatically discarded.

As in other tunicates, a consecutive set of anterior to posterior contractions (those beginning at the anterior or hypobranchial or branchial pole) is termed *advisceral beating* or an *advisceral cycle*, and the set of posterior to anterior pulsations (those beginning at the posterior or visceral pole) is called *abvisceral beating*. The encasing pericardium itself was never observed to contract in any of the tunicates examined in this study.

In general, only complete *advisceral* and *abvisceral* cycles were recorded. That is, counts of heart beats were begun only after observing a reversal and then all the beats in a given direction (pulsation series or cycle) were counted. Whenever possible (about 95% of the cases), a minimum of 3 complete consecutive pulsation cycles in both directions were recorded for each animal used. Where the size of the tunicates is not given, the animals measured at least 25 mm in body length (from the top of the siphons at the anterior end to the approximate beginning of the stolon). In most surgical experiments, the animals were not observed for more than 2 hours. Semi-isolated and isolated hearts were sometimes observed for 1 to 2 hours only, but often for less than 1 hour.

RESULTS

1. *The anatomy of the heart*

The heart is a long, relatively large, slightly twisted, dorso-ventrally oriented, unchambered, C-shaped, tubular vessel on the lower right side of the tunicate (Fig. 1, H). The heart opens into the hemocoel at each end (Berrill, 1961). The hemocoel is a large space of cavities and discrete vessels. The vessels were never observed to contract. Although difficult to see in both living and fixed material, single slit obovate ostia (Fig. 3, O) occur at each pole of the heart. The heart lies underneath the transparent tunic and is external to the large branchial basket (Fig. 1, B). The heart is tapered abruptly at both ends. Near the center of the heart is a short permanent constriction. When seen ventro-facially from the endostyle, the anterior apex is obovate and ventrally directed. The anterior pole terminates near the posterior end of the pale yellowish endostyle (Fig. 1, E). The posterior end of the heart is located internally, more deeply within the body than the anterior end, and is attached some distance to the right of the centrally-located, dark yellowish-brown stomach (Figs. 1 and 4, S). The stomach opens into the intestine on the ventral side. The intestine proceeds ventrad and then spirals around to the dorsal side, outside of the branchial basket (Fig. 1, IN). The tip of the posterior pole of the heart and its attachments was never seen as clearly as those of the anterior pole.

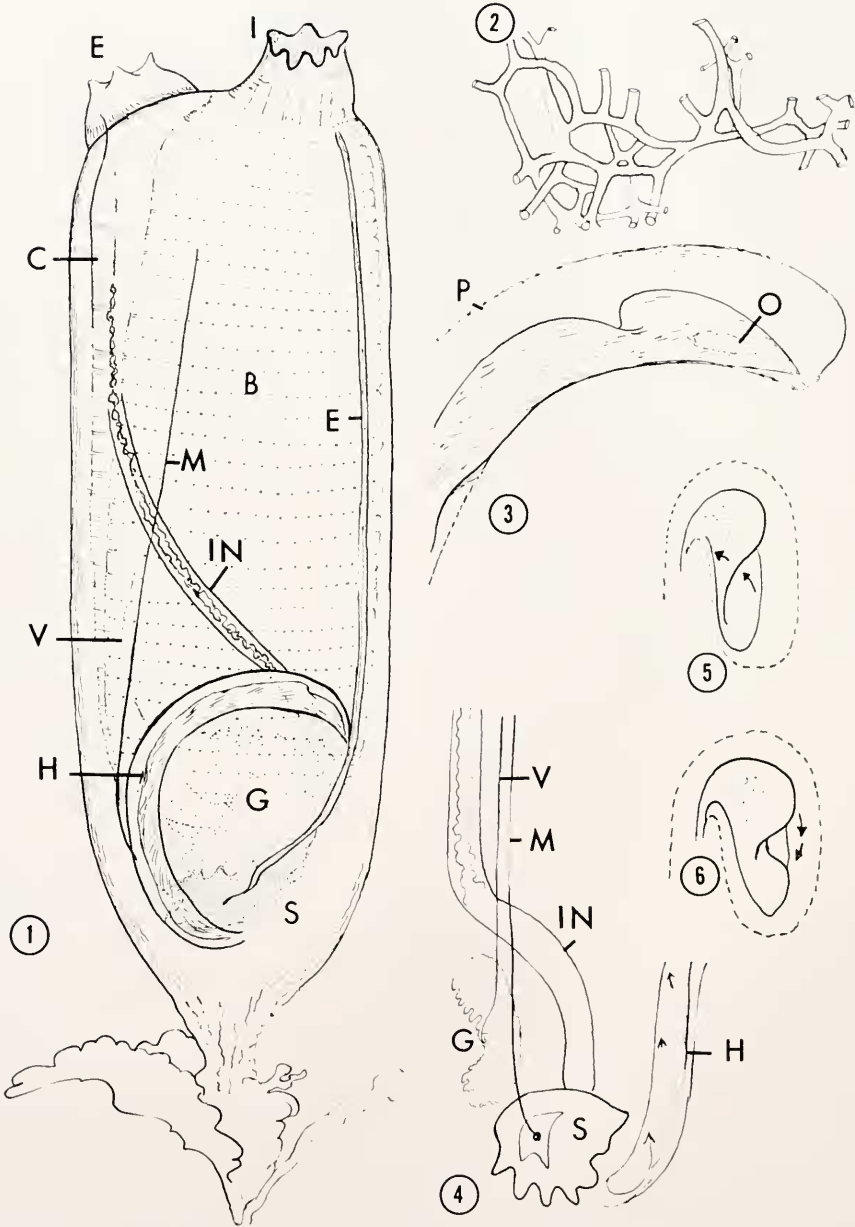


FIGURE 1. Semi-schematic view of adult *Ecteinascidia turbinata* as seen from the right side and showing the antero-dorsally located excurent siphon (E), the antero-ventrally located incurrent siphon (I), circular smooth muscle bands of the mantle (C), the large branchial (or pharyngeal) basket (B), the long, slender, ventral endostyle (E), the mucus column (M), the intestine (IN), the vas deferens (V), the heart (H), the gonads (G), and the stomach (S).

FIGURE 2. Non-contractile vessels within the tunic (semi-schematic).

The heart is completely encased in a large clear pericardium (Fig. 3, P). The pericardial cavity is probably the only remaining vestige of a coelom in tunicates (Barrington, 1965). While a definitive pericardial body was never observed, a large clump of hemocytes was sometimes seen within this cavity. During the formation of the heart, the pericardium folds inward and the site of the infolding subsequently serves as the sutural attachment along nearly the whole length of the heart (on its internal or left side, along the posterior margin). This long suture is the raphe (Fig. 7, R). *Ecteinascidia* totally lacks an epicardium, according to Lefevre (1897).

A large variable space exists between the anterior face of the heart and the anterior surface of the pericardium (Fig. 3). The heart is made up of a complexly wrapped single layer of long, thin, cross-striated muscle fibers (Fig. 7, H). The latter have a distinct spiral orientation. Complex folds are seen at the anterior end of the heart. When the isolated heart is examined in a fresh sea water whole mount with phase contrast optics, clear isotropic and grey anisotropic bands in the different fibers are seen to be aligned and are approximately equal in size. Sharp Z bands are present. A single cardiac muscle strand is made up of many nucleated cells connected to each other by several delicate, longitudinally orientated and banded rami. The muscle nuclei are large and ovoid, and each has a single conspicuous nucleolus. In all of the material examined, there was no indication that cardiac muscles degenerate or are cast off into either the heart lumen or the pericardial cavity [as in *Ciona* (Millar, 1953)].

In freshly dissected whole mounts seen with phase microscopy, long, fine (about 0.5μ in diameter in a large tunicate), phase-dark nerve fibers were observed in delicate web-like nets in the wall of the pericardium and around the heart (Fig. 8, N). These fibers lacked nuclei and had many small bead-like swellings or tiny nodes. Some of the many delicate arborizing branches were found within the myocardium itself. The nerve network was not localized or concentrated at any particular region of the heart. Some branches of the network could be traced to the ganglion located between the siphons at the anterior end of the animal (= "brain" ganglion). The nerve network did not stain when either intact tunicates or fresh whole mounts of the heart were kept in various concentrations of methylene blue in sea water for long periods.

2. General behavior of the intact heart

The heart of intact, highly responsive and healthy-looking tunicates exhibited a wide range of behavior. The most striking phenomenon was the condition of

FIGURE 3. The anterior end of the heart showing the large non-contractile pericardium (P), and the slit-like ostium (O). The ostium is shown more clearly than it is ever visible in fresh material (semi-schematic).

FIGURE 4. Dorsal schematic view of a portion of *Ecteinascidia* showing the vas deferens (V), mucus column (M), intestine (IN), gonads (G), stomach (S) and the heart (H). Note that the posterior pole of the heart is located alongside the stomach. Arrows indicate the pathway of peristaltic contractions during abvisceral beating.

FIGURE 5. Antero-ventral view of the anterior end of the heart during abvisceral beating. Note that the heart folds dextrad (arrows, semi-schematic).

FIGURE 6. Antero-ventral view of the anterior end of the heart during abvisceral beating, just as the contraction wave is reaching the anterior pole. Note that the heart is again folding dextrad (arrows).

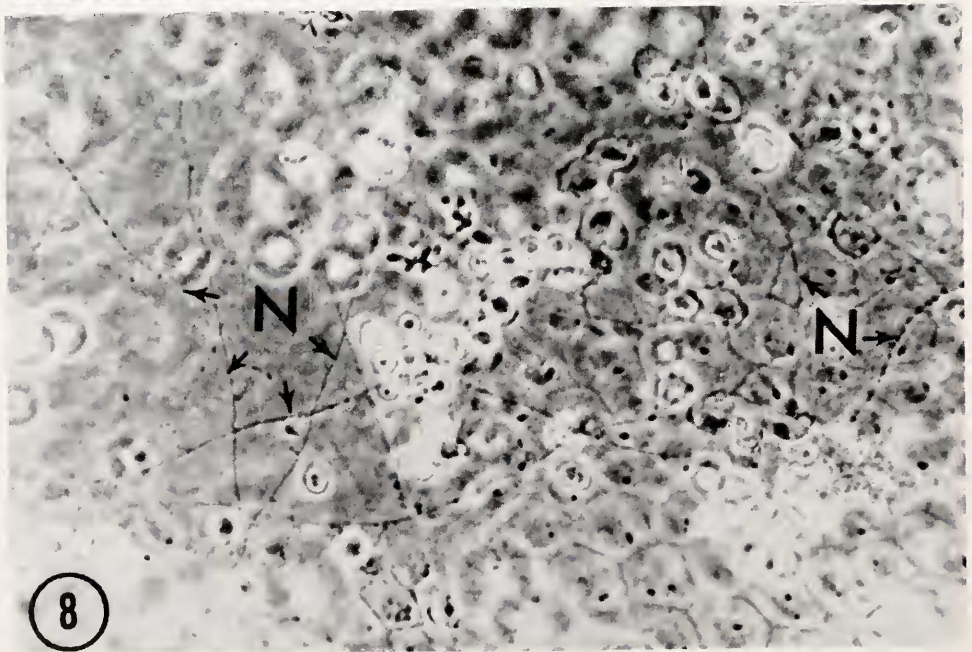
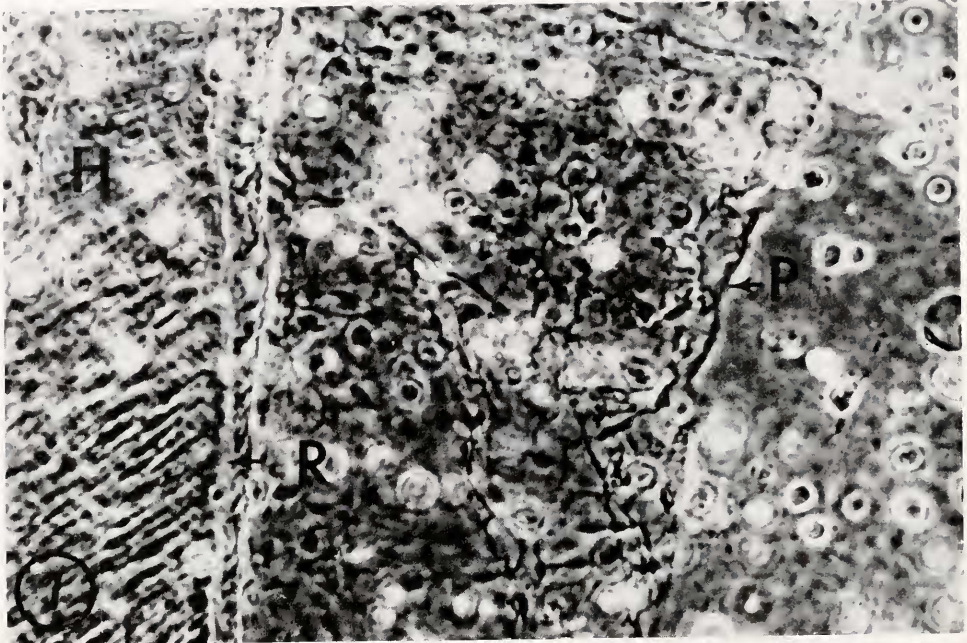


FIGURE 7. Unstained, phase-contrast appearance of a portion of the heart (H) of *Ecteinascidia turbinata* showing the raphe (R), and the pericardium (P). The numerous scattered globular structures are hemocytes.

FIGURE 8. Unstained phase-contrast view of nerve network (N) within the vicinity of the heart; note the fine beaded appearance of the nerves.

prolonged cardiac arrest in diastole. While this condition was commonly found in tunicates that had just been isolated from a colony, some hearts in diastolic arrest were observed continuously for as long as 2 hours without the occurrence of any pulsations. Arrested hearts were seen in many fully relaxed, normal-looking tunicates with active siphons. A few of these individuals were examined once a day over a period of several days so that in some cases, at least, the condition of cardiac arrest may be very prolonged in some otherwise healthy-looking and highly responsive animals (their siphons giving quick responses to touch). In most cases, if the heart was not beating within the first hour, it was not re-examined.

In some tunicates, the condition of diastolic arrest was followed by a state of prolonged erratic beating of the heart. Erratic beating was particularly noticed in animals which had just been isolated for study. In the commonest type of erratic heart activity, the heart would pulse one or two times in one direction or the other and then stop and start again at very irregular intervals. Prolonged erratic heart beats were observed in numerous contracted and relaxed tunicates. Erratic activity also included those cases in which one or both poles of the heart would contract locally: that is, heart contractions were not conducted over the full length of the cardiac tube. Local polar contractions were often rhythmic and sometimes alternated between poles, but generally these localized polar contractions were highly arrhythmic and not specifically alternative in character. Erratic cardiac activity also includes those cases where contractions would begin at both poles simultaneously or nearly so and be conducted towards the center of the tubular heart. Such bipolar beating was not a commonly observed phenomenon. More rarely, a third condition was observed in intact tunicates; a single contraction would begin in or near the constricted center of the heart and sweep outwards towards the poles.

Over a 5 year period, the condition of prolonged cardiac arrest was found in about 5% of the healthy-looking individuals examined. The condition of prolonged erratic beating was seen in 2% to 39% (average of 18.4%) of the individuals. The majority of the tunicates had hearts which exhibited prolonged rhythmical beating. Rhythmical beating sometimes followed a period of prolonged erratic cardiac activity. Rhythmical beating of the heart in intact *Ecteinascidia* proved to be highly variable. No differences could be seen between the degree of distension of the heart within the pericardial cavity and any specific cardiac activity in intact animals.

3. Heart contractions, reversals, and circulation of the hemolymph

The heart's contractions and circulation of the hemocytes within the clear hemolymph can be seen in its finest details and with brilliant clarity in most intact *Ecteinascidia*. When the heart beats, a peristaltic wave normally begins only at either the anterior or posterior pole. Normally, the contraction waves appear as large, full, ballooning billows that sweep over the tube. On one occasion a tightly constricted heart tube was seen in an intact healthy-looking individual; this animal's heart beat and reversed very rapidly. Later, this animal's heart expanded fully, and did not then reverse as frequently.

Heart contractions are conducted in definite twisting spiralling patterns over the length of the heart (see, for example, arrows in Fig. 4). During contractions,

the heart folds dextrad (see Figs. 5 and 6). Only a relatively small portion of the heart tube (about one-twelfth of the tube length) is partially (but never completely) occluded during the passage of a constriction wave.

As in all other tunicates thus far studied (see Krijgsman, 1956), after a variable number of beats the direction of the contractions changes from one pole to the other. In *E. turbinata* these reversals begin in the late embryo (they are not synchronized with those of the mother) and continue throughout the life of the individual. Although repeated specific synchronized reversals of the heart in two connected individuals have been seen among connected samples of *Ecteinascidia conklinii typica*, synchronized reversals have never been observed between connected individuals of *E. turbinata*.

Reversal in direction of peristaltic waves occurred with and without pauses of generally short but highly variable duration between sets of beats. During a reversal pause, the heart tube may passively swell and subside slightly, and very fine ripples may pass over the non-contracting vessel. Pauses between consecutive beats in both advisceral and abvisceral directions are quite common before the heart beat reverses. As in *Ciona*, the heart rate is usually faster shortly after heart reversal than near the end of a pulsation series.

Towards the end of a given cycle, the contractions tend to become slower. One or more final beats may still occur before the heart stops and reverses. Many times the heart stops very suddenly without slowing down, and reversal in direction occurs after a very brief pause. Pauses before reversals varied enormously (from about 0.5 seconds to far more than 15 seconds); no definite relationships between these pauses and the size of the animals were noted. In a few individuals, at the very end of a cycle, there may be an obvious sudden brief backflow of hemolymph at the excurrent ostium. The actual backflows seen at the anterior end of the intact heart never exceeded one-seventh the length of the heart tube.

When heart pulsations cease, the entire circulation in the whole animal also instantly ceases. In intact animals during rhythmical beating, hemocytes can be easily seen flowing in dense spiralling streams into and out of the heart through the slit ostia at each pole. The heart never ejects all of its hemolymph into the extracardiac system *in vivo* at any one contraction. When the heart and pericardial sac are removed from the animal without any mechanical injury to these structures, they both always collapsed and were largely empty of hemolymph. The heart is filled from ostia at the poles; how the pericardium is filled is completely unknown.

During advisceral beating, the hemolymph moves away from the animal's incurrent (ventral) siphon and passes posteriorwards in two major currents down the sides of the endostyle. The hemolymph in the many small branchial vessels circles around the body and flows into the endostylar currents. Numerous close observations of the vessels in the test and branchial basket never showed them to contract in any way. Hemolymph flows from the posterior end of the heart and is directed into at least four channels. One of these streams goes around the non-contractile stomach towards the gonads on the left side of the animal. Another stream passes upwards towards the excurrent (dorsal) siphon and forms distinct rivulets on either side of the non-contractile intestine. Still another stream moves into channels along the esophagus and mucus column and proceeds towards the siphons and branchial vessels.

During abvisceral beating, one large current of hemolymph flows away from the excurrent siphon posteriorwards and passes over the stomach in a direction opposite to that during advisceral beating. Hemolymph streams pass posteriorwards over the intestine. Hemolymph spurts out of the anterior end of the heart and anteriorly-directed currents flow up to the incurrent siphon. At the same time, one large posteriorly-directed current of hemolymph flows on either side of the endostyle.

4. Variability of the heart rate

By far the most striking feature of the contractions of the heart in intact *E. turbinata* was the extreme variability in the number and duration of both advisceral and abvisceral beating. The amount of hemolymph circulated during a particular pulsation series would therefore vary enormously even in the same individual. A long series of studies were made to determine some of the possible sources for the great variability of the heart rate in normal animals.

A few records were obtained from 16 newly emerged tadpoles by placing them between a slide and coverslip in a drop of fresh sea water. No heart beats could be detected over a 5 minute interval in three of the individuals. The hearts stopped beating in 4 other individuals which were being examined. The heart rates of the tadpoles examined were highly variable. The number of beats occurring in both advisceral and abvisceral cycles were obtained along with the duration of each. The number of beats in advisceral cycles varied from 1 to 56 and averaged 14.3. The duration of this cycle varied from 5 to 67 seconds, with a mean of 24.6 seconds. The number of beats in the abvisceral cycles varied from 2 to 87, with an average of 21.0 beats. The duration varied from 4 to 222 seconds, with a mean of 42.5.

A single large (25 mm) healthy-looking tunicate was selected from a colony on the day of its capture and was arranged in a vertical position with its siphons uppermost at the bottom in a 100 ml graduate and was examined continuously over a period of 7 hours under varying amounts of fresh sea water. No marked differences in the number of heart beats and their duration were apparent in this individual in differing volumes of sea water. Similar results were obtained in contrasting heart rates of another individual kept first in 250 ml then in 50 ml of fresh sea water over a 3 hour period. The next day this same tunicate was gently removed from the 100 ml graduate and placed on its right side in a small dish containing 10 ml of fresh sea water. After this change, the tunicate was examined: its heart had stopped beating. The heart began to beat erratically and did so for at least 10 minutes. After this, the heart began to beat rhythmically again at a rate not strikingly different from that in the vertical position in 90 ml sea water.

Complete heart rates were taken from 194 individuals that ranged in size from about 1.5 to 40 mm in length. No clear-cut relationships or trend between the size of the tunicates and heart beat and its variability could be seen.

Although the heart rate of *Ecteinascidia* is highly variable between individuals, the heart in 5 groups of 261 animals averaged 60 to 77 beats in either ad- or abvisceral direction (overall mean 69.6 ± 1.6 beats) each summer over a 5 year period. The average duration of beating in either ad- or abvisceral direction

ranged from 87 to 114 seconds (overall mean 99.5 ± 2.6 seconds.) The overall advisceral rate for each of 5 summers was 71.4 ± 2.7 beats in 102.5 ± 4.4 seconds and the overall abvisceral rate was 67.9 ± 2.5 beats in 96.4 ± 4.1 seconds. Thus, there is no significant difference (95% confidence limits) in rates from either direction. In different words, neither the anterior (=hypobranchial) nor the posterior (=visceral) center is dominant in normal intact healthy *Ecteinascidia*.

Daily mean heart rates from 70 relatively large tunicates (20 to 30 mm length) from a single colony were analyzed in relation to days (1-12) they were kept in the sea table. The mean advisceral rate was 66.6 ± 5.0 beats in 93.1 ± 5.0 seconds, and the mean abvisceral rate was 62.3 ± 4.4 beats in 87.5 ± 5.0 seconds. No obvious trends in the heart rates were apparent relative to time after captivity.

Three tunicates were placed in small dishes containing 10 ml of sea water and three complete advisceral and three complete abvisceral rates were recorded consecutively every hour on the hour for a 24 hour period. The sea water was renewed at aperiodic intervals. The first readings were compared with those in subsequent hours. The heart rates were more variable during the first hour than at any time thereafter among all three individuals. After the first hour, the heart beats and their duration tended to be remarkably uniform within a single individual (most rates were within 10 beats of each other per individual). Great differences were still apparent, however, between heart rates among the three individuals. Nevertheless, in small amounts of unchanged sea water, the hearts maintained a strikingly uniform number of beats for many hours. In all three tunicates the heart rate began to increase around the tenth or eleventh hour: that is, the number of beats and their duration in both advisceral and abvisceral cycles definitely increased with time, and this increase was well-established around the twelfth hour. In unchanged sea water the heart contracted more slowly and beat longer in each direction during the last 12 hours than during the first 12 hours.

Three fresh tunicates were then placed in small dishes containing 10 ml of sea water which was constantly changing at a rate of 2.5 to 17 ml per minute, and heart rates were taken hourly over a 24 hour period. The number of heart beats in each tunicate decreased after the first hour. The number of beats was strikingly uniform within and between all three of these individuals for about 20 out of the 24 hours. While the number of beats was not highly variable, the duration of both cycles was extremely variable. During the last 4 hours, the duration of both cycles definitely increased. In marked contrast to the tunicates in unchanged sea water, the number of heart beats did not significantly increase at any time during the 24 hour period when the sea water was constantly flowing. Although the duration of both cycles increased in changing sea water, this increase was much less and occurred considerably later than in tunicates kept in unchanged sea water.

In summary, the variability of the heart rate of *E. turbinata* is very great during the first hour of observation. This variability is not related to orientation of the tunicate in the observation chamber, to the volume of sea water in the observation chamber, to the body length, or to the time held in the sea table, or to the use of animals in obviously poor physical condition.

6. *Studies on heart reversals in intact animals*

In manipulating tunicates for heart rate studies, it had been qualitatively observed that handling frequently was associated with erratic beating and with greatly increased reversal rates. Therefore, two experiments were made to test the effects of external pressure on the heart rates of intact tunicates. In the first experiment 5 tunicates were used to obtain pre-test heart rates in fresh sea water and then a uniform weight was positioned on the body to exert a strong continuous pressure on the upper halves of the animals, considerably anterior to and outside of the heart region. This pressure caused violent contraction of the tunicates and tight closure of both siphons, resulting in an increased pressure in the hemocoel. A series of heart rates were taken during the period of continuous pressure and then the weight was suddenly lifted with a consequent sudden marked decrease in pressure in the hemocoel, and another series of heart rates were taken as soon as

TABLE I

Mean heart rates with standard errors from Ecteinascidia turbinata before, during, and immediately after strong external pressure on the anterior ends of five animals

Treatment	Advisceral rates		Abvisceral rates	
	Beats	Seconds	Beats	Seconds
No pressure	93.1 ± 7.9	115.1 ± 18.5	84.9 ± 11.7	112.2 ± 12.4
Strong pressure	68.5 ± 16.3	85.2 ± 20.1	59.2 ± 10.8	88.5 ± 15.2
After pressure	96.1 ± 15.6	100.7 ± 23.7	73.7 ± 17.3	94.8 ± 22.3

possible after the pressure was released. As summarized in Table I, strong pressure on the anterior ends of intact tunicates increased the already great inherent variability of cardiac data.

Five tunicates were used to study the effect of localized pressure on the heart in intact animals. A blunt probe was firmly and strongly pressed over the anterior end and/or over the center of the heart immediately after the first 5 beats of either the advisceral or abvisceral cycle. The probe could not be placed or maintained firmly or specifically directly over the abvisceral end because it is located much more deeply within the body of the animal. The data in Table II show that external pressure over the heart tube greatly increases the rate of reversals in intact animals. After external pressure, the hearts averaged 18.5 beats in 32.4 seconds before reversing in direction. It was observed that even during strong external pressure over the anterior center, advisceral beats would still arise therefrom.

The siphons, along with the "brain" ganglion (which is located anteriorly between the siphons), were removed with single scissor cuts from 5 tunicates shortly after taking a series of pre-test heart rates, and heart rates were recorded immediately afterwards and one hour and 3 hours afterwards. The heart rates were highly variable within and between individuals before (means of 66.5 ± 9.5 beats in 93.4 ± 13.7 seconds) and after siphonal removal (means of 75.5 ± 3.3 beats in 107.8 ± 5.8 seconds). The hearts continued to beat and reverse for at

TABLE II

Effects of external pressure after the first 5 beats in a given direction on reversals of the intact heart of Ecteinascidia turbinata

Animal No.	Trial No.	Heart rates before reversal after pressure on					
		Anterior center at beginning of advisceral cycle		Center of heart at beginning of advisceral cycle		Anterior center at beginning of abvisceral cycle	
		Advisceral		Advisceral		Abvisceral	
		Beats	Seconds	Beats	Seconds	Beats	Seconds
1	1	22	33.8	21	31.2	10	14.2
	2	28	42.0	—	—	19	25.0
2	1	22	42.4	30	53.6	31	58.2
	2	25	44.2	—	—	32	60.0
3	1	11	23.2	18	35.0	19	35.0
	2	16	32.2	—	—	16	30.6
4	1	9	17.0	19	30.0	18	33.0
	2	8	13.8	—	—	17	28.4
5	1	14	25.6	14	25.2	19	33.0
	2	8	14.8	—	—	17	29.6

least 24 hours after siphonal (and brain) removal from a series of representative tunicates.

Ten tunicates were used to study the effects of cutting through the approximate center of the intact heart. After a series of pretest rates were taken, each heart was cut completely through with a single small scissor cut. The incision always led to sudden brief loss of a variable (usually large) amount of hemolymph which clotted almost instantly. The wound was sealed with great rapidity and then blackened. This rapid clotting would seriously interfere with accurate blood volume estimations based on attempted exsanguination. The hearts always stopped in diastole the instant they were cut with the scissor blades. Unlike *Ciona*, the cut hearts did not swell. Although small and large amounts of hemolymph could be removed from the heart *in situ*, no marked differences in the diameter of the intact heart could thus be consistently produced in *Ecteinascidia* (unlike *Ciona*). The heart was never observed to distend to such an extent as to fill the large pericardial cavity in any of numerous surgical experiments. After cutting the center of the heart, the tunicates were observed for periods ranging from 25 minutes to 20 hours; the average time was 105 minutes. The hearts remained in diastolic arrest for 1 minute to more than 2 hours, for an average of about 20 minutes. In 80% of the tunicates the posterior half began to beat before the anterior half, and, in some cases, was the only half to beat at all. When the anterior half did beat, the contractions were usually less vigorous than those in the posterior half. In general, the contractions were only towards the cut center. In a few cases, beats were initiated from the site of the incision. Re-

versals only rarely occurred in centrally-cut hearts. When contractions occurred in both anterior and posterior halves in the same tunicate, they were very rarely synchronized. Centrally-severed hearts were never observed to resume truly normal rates with periodic reversals like those of untreated controls.

When the heart was removed along with the pericardium in a series of tunicates, it collapsed. While a few pulsations sometimes persisted from one or both poles of the isolated heart, the contractions were highly abnormal in character. Usually individual muscle fibers would contract either in unison and/or asynchronously with other heart muscle fibers. Most attempts at filling the excised heart and/or the pericardial sac with sea water were not successful. Where the excised heart and/or pericardial sac could be inflated to various degrees, however, either the heart never beat at all or it pulsed very erratically and abnormally. That is, the contractions were not smooth, large, full, billowy waves, but peculiar vibratory contractions of small amplitude. Periodic mechanical stretching and releasing of isolated and semi-isolated (exposed) heart preparations did not induce beating in already arrested hearts and generally appeared to stop erratically beating hearts. Gentle probing or touching of quiescent isolated or semi-isolated (exposed) hearts generally did not elicit rhythmic contractions, although one or many individual cardiac strands would contract.

DISCUSSION

The present studies on *Ecteinascidia* are interpreted to mean that under optimum conditions, the heart of a given individual will beat and reverse at a uniform, if highly idiosyncratic rate, whether the animal is an embryo, a tadpole, an immature zoid or a mature adult. If the animal is mildly disturbed by mechanical stimuli, then its heart rate often rapidly becomes highly variable with a tendency to be accelerated for about one hour. If the disturbance is stronger, then the heart beats erratically for a variable but usually long period, and if the disturbance is still greater, then the heart stops beating completely for a variable but generally long time. What could be the advantage of no circulation during stress? Although one advantage might be that it would reduce hemolymph loss, the hemolymph rapidly clots *in vitro* and quickly seals even large wounds. It would be useful to know if cessation of circulation would aid wound healing.

Ecteinascidia may look perfectly normal, have its siphons open and be highly sensitive to touch, and live for a long time without a pulsating heart (*i.e.*, with no circulation of the hemolymph). That a relatively large number of such individuals may have a very erratic circulation of the hemolymph indicates that a heart and efficient rhythmical circulation of the blood are not essential. This interesting condition is like that described for certain insects (Jones, 1964).

Since the fine network of nerves within the pericardium and myocardium of *Ecteinascidia* is so diffuse, the nerves cannot be acting directly and specifically on the poles from which all of the beats normally arise. Thus, it is believed that the nerve network cannot be associated with the initiation of heart beats. The *Ecteinascidia* heart definitely lacks nerve cells ("cardiac ganglia"). Almost all workers agree that the tunicate heart possesses essentially myogenic pacemakers. Although arthropods are very often said to have "neurogenic" hearts, this is certainly not true for the insects, which represent the largest class of these animals

(see, for examples, Jones 1954; McCann, 1961; and Miller, 1969). Most insects are believed to have innervated myogenic hearts (Jones, 1964). Thus, the *Ecteinascidia* heart has more in common with the typical insect heart than with the typical neurogenic hearts of *Limulus* (Carlson, 1905) and many Crustacea (Maynard, 1960).

It is assumed that the extensive nerve network in and around the heart has a marked influence on the heart beat and reversals in *Ecteinascidia*. Perhaps it is the source of the extreme sensitivity of the heart: the heart can very quickly accelerate, decelerate, and stop and start again. Under optimum conditions, it is probably exquisitely well-regulated, although this system is very easily disrupted. Since the *Ecteinascidia* heart continues to beat and reverse normally when the "brain" ganglion has been removed, the network cannot be controlled or regulated by this ganglion.

Specific findings on *Ecteinascidia* can be compared best to those available for *Ciona*. While the *Ecteinascidia* heart is well-innervated, there are conflicting reports on the question of cardiac innervation in *Ciona*. Thus, Krijgsman (1956) did not believe the *Ciona* heart was innervated. Millar (1953), Ichikawa (1966) and Anderson (1968) did not find cardiac nerves in their studies on *Ciona*. On the other hand, Alexandrowicz (1913, page 373) observed many fine cardiac nerves in the myocardium of *Ciona*. Although Florey (1951) specifically cites the work of Fedele (1923a; 1923b; 1927) in connection with his proposal that cardiac nerves in *Ciona* were cardio-inhibitory, I can find no statements concerning either the heart or cardiac nerves in Fedele's papers. Subsequently, however, Florey (1966, page 215) felt that tunicate (*i.e.*, *Ciona*) hearts lacked "nervous control." Bone and Whitear (1958) described a plexus of nerves in the wall of the pericardium of *Ciona*, and stated the nerve fibers ran along the length of the heart. They suggested that these nerves were sensory. Apparently they did not see any branches of the nerve to the heart wall itself. Markham (1958) also observed cells evenly scattered along the suture of the *Ciona* heart which stained with methylene blue. He presumed these cells to be sensory. Kriebel (1968c) found the *Ciona* heart to have cholinceptive properties, and he felt (1968a, page 450) that there could be an intrinsic regulation of the heart beat frequency. He maintained (1968a), however, that removal of either the raphe or the undifferentiated line from the isolated *Ciona* heart did not prevent heart reversals.

While Kriebel (1968a) found that some exposed-but-*in-situ* hearts of *Ciona* could beat for 8 hours without reversing, this was never observed in *Ecteinascidia* in any type of preparation (intact or experimental). Although Kriebel reported that body wall stimulation in *Ciona* increased the number of heart beats and decreased the reversal rates, this could not be statistically demonstrated in *Ecteinascidia*. Perhaps the level of external stimulation was not the same; the level was not precisely regulated in *Ecteinascidia*. According to Skramlik (1938) and Bacq (1934), the anterior (branchial) center of the intact *Ciona* heart has a greater frequency than the visceral center; according to Kriebel (1968a), this difference is evident at the beginning but not at the end of a pulsation series in isolated hearts. In *Ecteinascidia*, however, no statistically significant differences in total number of beats in a given cycle or their duration from either pole could be

demonstrated in intact animals. Nevertheless, there is a tendency for the beats to be faster at the beginning than near the end of a given cycle in *Ecteinascidia* as in *Ciona*. In *Ciona* the heart rate is said by Kriebel (1968a) to depend upon the size of the tunicate. This could not be shown to be true for *Ecteinascidia*.

The heart of *Ecteinascidia* appears to differ in a number of other ways from that of *Ciona*. Thus, the *Ecteinascidia* heart does not swell at all after the pericardium is punctured, whereas in *Ciona*, the heart swells and fills the entire pericardial space (Kriebel, 1968a). While Kriebel (1968a) stated that he was able to abolish arrhythmic heart activity in intact *Ciona* by stimulating the animals to contract, erratic heart activity in *Ecteinascidia* was never thus abolished; in fact, causing them to contract often led to prolonged arrhythmia.

While Kriebel (1968a) found that 70% of the hearts of *Ciona* contracted irregularly when first isolated, 99.9% of the hearts of *Ecteinascidia* stopped beating altogether when first isolated. While 25% of the isolated hearts of *Ciona* would continue rhythmical beating after being cut in half (Kriebel, 1968a), none of the isolated hearts of *Ecteinascidia* beat rhythmically when so treated. In *Ciona*, Kriebel (1968a) found that collapsed and overdilated hearts could beat in only one direction; this phenomenon was never observed in *Ecteinascidia*.

There is no doubt that pressure directly over the heart of intact *Ecteinascidia* significantly increases the number of reversals. In *Ecteinascidia* this is probably effected via the nerve network. Although external pressure over the heart does lead to an increase in reversals, this does not mean that back pressure within the system can account for reversals as Florey (1966) proposed for *Ciona*. Thus, strong pressure on intact *Ecteinascidia* some distance away from its heart and the sudden release from this pressure did not elicit specific heart reversals. Also, the sudden loss of a massive amount of hemolymph, when the siphons were cut off, did not alter the reversal rate. Furthermore, strong continuous pressure directly over an inactive pacemaker did not prevent beats from originating within it.

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SUMMARY

1. The non-chambered tubular heart of the colonial ascidian *Ecteinascidia turbinata* is made up of a single layer of very long, thin, cross-striated and spirally-wrapped muscles. Enclosed within a large non-contractile pericardial sac, the heart and pericardium are innervated by a fine non-ganglionated network. The channels and vessels through which hemolymph circulates are non-contractile.

2. In recently captured, intact, and healthy-looking tunicates over a 5 year period, the heart exhibited either prolonged diastolic arrest, prolonged highly erratic (arrhythmic) beating, or fairly rhythmical but highly variable beating with periodic reversals in beat direction. No significant differences were found in contraction rates originating from either pole of the heart.

3. The heart begins to beat and reverse in the embryo and reversals continue throughout life. Synchronous reversals were not observed either between embryos from the same mother or between two or more attached individuals.

4. The extremely variable character of rhythmical heart beating involves both the number and duration of complete advisceral and abvisceral cycles. The variability is not due to (a) developmental stage (embryo, tadpole, mature individuals), (b) body length, (c) time in sea table after captivity, or (d) amount of sea water in examination chamber.

5. The great variability of heart rhythmicity in intact animals diminishes markedly within the first hour after isolation, if the tunicates are left undisturbed. The heart is capable of beating and reversing with remarkable uniformity for long periods both in unchanged and in constantly changing sea water in small chambers. Simple manipulation of intact tunicates can readily and quickly induce renewed and extreme cardiac variability.

6. Pressing externally directly over the anterior (advisceral) pole of the heart or over the center of the heart shortly after the beginning of either the advisceral or abvisceral cycle significantly increases the rate of heart reversal.

7. Hearts continue to beat and reverse for a long time after both siphons (including the "brains") are removed. When the pericardium alone of the heart itself is punctured with a fine needle or cut with scissors, the heart always stops instantly in diastole for variable periods of time. The two halves of a centrally-severed heart are capable of reversing. Heart reversals were observed after puncturing small portions of the anterior and posterior poles of the heart. When the anterior pole was completely ablated, heart beats were observed only from the posterior (abvisceral) pole. Semi-isolated hearts were never observed to beat normally and rhythmically.

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